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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) Currently we lack information on the role of the men-only chromosome, the Y chromosome, on prostate cancer. This project is designed to address this problem and to identify candidate genes on the Y chromosome involved in this male-specific cancer. The objectives are: 1) to study the expression of Y chromosome genes in prostate cancer and 2) to evaluate their effects in over-expression in the prostate of transgenic mice. We have completed a survey on the expression of 31 Y chromosome genes in prostate cancer and have identified TSPY gene to be the most likely one to play a role in male oncogenesis. We have isolated and sequenced several polymorphic TSPY transcripts in normal and cancerous prostate samples, demonstrated that over-expression of TSPY potentiates cell proliferation in vitro and tumor formation in vivo. TSPY interacts with the mitotic cyclin B. Its expression is influenced by androgen. For the no-cost extension period, we hope to finish our transgenic study in over-expressing this and other Y chromosome gene(s) in prostates of mice and to evaluate its effects in prostatic oncogenesis and to define the TSPY domain(s) important for its interaction with the mitotic cyclin B. These studies will provide important information on the role of this male-specific chromosome on prostate cancer development and treatments.				
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## INTRODUCTION

The male-specific chromosome, Y chromosome, plays critical roles in the physiology of male organs, such as testis and prostates. Abnormalities or mutations among genes on this chromosome are expected to have significant influences on disease development, such as infertility, and men-only cancers of the testis and prostate (1). There are two specific objectives for the present project. The first one is to identify the most likely candidate proto-oncogene(s) among the genes on this chromosome using a series of expression studies. The second one is to evaluate the role(s) of the identified candidate(s) in oncogenic activities in transgenic mice, particularly on the prostate cancer. These studies should provide valuable information on the potential role of the human Y chromosome in prostatic oncogenesis.

## BODY

### *Task 1. To identify the candidate gene on the Y chromosome that plays a role in prostate cancer*

Studies under this task have already been accomplished during Year 1 and 2. The results have been communicated in reports of the previous years of this project. Briefly, we had studies a total of 31 genes on the Y chromosome in a panel of prostatic samples consisting of normal and cancerous tissues. The results identified one strong candidate gene, TSPY that is expressed at high levels in prostate cancer. Its expression increases with increasing degrees of malignancy (2). TSPY is also a candidate gene for the gonadoblastoma locus on the Y chromosome (GBY) that predisposes the dysgenetic gonads of XY sex-reversed females to cancer development (1,3,4).

### *Task 2. Functional evaluation of Y chromosome genes in prostate cancer*

The identification of a strong candidate gene, TSPY, from the Y chromosome that may play a role in prostatic oncogenesis has led us to focus on defining the probable function of its over-expression in prostate cancers. Previous studies under this task further demonstrated that over-expression of the TSPY gene potentiates cell proliferation in culture and tumorigenesis in nude mice (5). Studies on its expression profile in prostate cancer demonstrated that there are polymorphic forms of this gene, resulting in a spectrum of TSPY variant proteins. All TSPY variant proteins harbor a conserved domain homologous to those present in a family of cyclin B binding proteins, such as NAP-1 and SET oncoprotein, involved in cell cycle regulation (6-9). These results are significant in terms of a potential role of cell cycle regulation of TSPY, abnormalities of which might be involved in the disruption of such regulatory mechanism in male tumor cells.

Our studies in Year 3 were focused on defining the possible interaction between TSPY and the mitotic cyclin B, its regulation by androgen, and on evaluating its role in prostatic oncogenesis in transgenic mice. They are outlined in the following paragraphs.

## TSPY INTERACTS WITH THE MITOTIC CYCLIN B

We have adopted an in vitro binding and pull-down assay to study the possible interaction between TSPY and cyclin B. Initially, the *Xenopus* cyclin A, B1 and B2 were subcloned into a GST expression vector and expression in bacterial hosts and purified from total cell extracts. The human TSPY cDNA was transcribed and translated in vitro using a TnT kit. TSPY is labeled with <sup>35</sup>S-methionine in the reaction mixture. To demonstrate an interaction, labeled TSPY proteins were reacted

independently with GST-cyclin A, B1 or B2 fusion proteins. The GST fusion and bound proteins were purified by affinity binding to glutathione-conjugated resins and analyzed by SDS-PAGE. Results from this study demonstrated that TSPY is preferentially retained by GST-cyclin B1 or B2 fusion proteins, but not by GST alone nor GST-cyclin A (Figure 1A). Similar studies with the human cyclin B confirmed that human TSPY did interact with the human cyclin B.

Previous studies demonstrated a spectrum of TSPY variant proteins in prostate cancer samples. To determine if these variant TSPY proteins do interact with the mitotic cyclin B similarly, we had subcloned the respective cDNAs of the TSPY proteins into an in vitro TnT vector. Radioactively labeled variant TSPY proteins were used in protein-binding assays with a GST- human cyclin B1 fusion protein. The results demonstrated that these variant proteins did bind to cyclin B1 and were co-precipitated with the cyclin B1 protein, but not GST alone (Figure 1B). Previous results suggested that alternative splicing events generate different transcripts deleting 92, 110 and 145 amino acids from the amino end of the predominant TSPY protein of 295 amino acids (Figure 2). These results suggested that the cyclin B binding domain is primarily located in the carboxyl end of the protein, as predicted by sequence alignment with NAP-1 and SET proteins. Although we are still uncertain on the functions of the different domains of the TSPY protein, deletions of portion(s) of its amino end might suggest a loss of certain function, assigned to this portion of the molecule while the cyclin B binding function is maintained in these shortened variants. It will be extremely interesting to define the functions of the different domains of TSPY and to evaluate what effects they may exert when they are deleted from the respective proteins.

#### TSPY PROMOTER AND ANDROGEN REGULATION

To evaluate the regulation of TSPY gene in prostatic cells, a cell transfection and promoter analysis strategy was implemented. Various portions of the human TSPY gene promoter were subcloned in a luciferase expression vector, such that the TSPY promoter directly regulates the reporter gene (Figure 3A). The expression of the luciferase was then assayed in a cell transfection assay. A variety of host cells were used in these studies. They included prostatic cells, LNCaP (androgen responsive) and PC3 (androgen non-responsive), GC1spg (spermatogonia-like) and GC2spd (spermatocyte-like), COS7, HeLa and NIH3T3 cells. Preliminary results from these studies suggested that only 160-bp upstream of the transcription start-site of the TSPY gene was sufficient in directing the expression of the luciferase gene in all cell types (e.g. Figure 3B). However, a bimodal expression pattern was observed in GC1spg, GC2spd and PC3 cells (Figure 3C, D, and E respectively). When additional upstream sequences (upto -2.4 kb) were included in the promoter, a reduction of the reporter activities was observed, suggesting that a repressor-like regulatory element(s) was present upstream of the core promoter (at -160 bp). Such expression pattern was absent in the HeLa female cells (Figure 3F). Since GC1spg and GC2spd and PC3 were derived from the testicular spermatogonial, spermatocyte and prostatic cells respectively, the bimodal regulation of the TSPY promoter in these cells suggested that they might resemble a regulatory mechanism in these male-specific cells, as compared to the more generic cells, such as HeLa cells.

TSPY expression was stimulated by androgen in the responsive cell line, LNCaP while its expression was not affected by the male hormone in the non-responsive cell lines, PC3 and COS7, even in the presence of a transfected androgen receptor gene. It will be interesting to determine the mechanism(s) by which TSPY is regulated by the male hormone that has been demonstrated to be important in the etiology of prostate cancer (10).

## TRANSGENIC MOUSE STUDIES ON TSPY GENE

Transgenic mouse studies are important components of the project. When targeted to prostate-specific expression, these studies can confirm the roles of the Y chromosome genes in prostatic physiology and diseases. For the past couple of years, we had implemented a tet-off transgene regulation system that allowed a manipulated expression or repression of the transgene in the host animals (11). This system constituted two components, a transactivator and a responder, by which the responder (target) gene could be regulated in bi-transgenic animals. We had successfully constructed various prostate-specific transactivator lines and responder lines. Currently, we are in the process of identifying the best combination of transactivator and responder lines for these experiments. Unfortunately, our progress had suffered somewhat with the unexpected departure of a key worker, Dr. Xing Xing Liu, in the early part of Year 3. Hence, this portion of the research was performed by the second postdoctoral fellow, Dr. Tatsuo Kido, who was also responsible for the protein and promoter work. To accomplish this and other aspects of the project, we had requested and been granted a no-cost extension of this project for 12 months.

We had also generated transgenic mice harboring the native TSPY gene. Three transgenic founders were obtained. Two of the transgenic founders showed extreme abnormalities in their stature (much smaller than non-transgenic littermates) and died within two months after birth. The third founder seemed to be mosaic animal and never passed on his TSPY transgene to its offsprings. Although inconclusive in nature, these results suggested that abnormal TSPY expression might be detrimental to the health/development of the transgenic animals. Hence, a manipulated expression of the TSPY transgene, e.g. using the tet-off system, might be essential for these experiments.

## FUTURE DIRECTIONS

For the no-cost extension period, we hope to achieve additional results in two specific areas. First, we plan to finish our transgenic mouse studies using the tet-off system directly expressing the TSPY gene in the prostates of transgenic mice. So far, using the same regulatory system, we demonstrated that cells over-expressing TSPY formed tumors faster than those repressing the TSPY gene in athymic nude mice. We anticipate that over-expression of TSPY specifically in the prostates of the hosts might have similar effects.

The second goal for this period is to define the cyclin B binding domain of the TSPY protein. As discussed above, variant proteins were observed in the prostate cancer samples. Some of them had deleted a major portion of the amino terminus portion of the predominant species. If the deleted portion harbors a major regulatory function, then these shortened versions of the TSPY protein might still bind to the mitotic cyclin B, but fail to perform the function(s) ascribed to the deleted amino domain. Hence, we plan to continue and to include site-directed mutagenesis techniques in the binding studies. Eventually, such in vitro studies will be significant in defining the functions of the TSPY variants in future transgenic mouse studies.

## KEY RESEARCH ACCOMPLISHMENTS

- Identify TSPY to be a strong candidate gene involved in prostate cancer
- Demonstrate the binding of TSPY protein to the mitotic cyclin B



- Demonstrate the expression of the shortened version of TSPY proteins
- Demonstrate a complex regulatory mechanism at the promoter of TSPY gene
- Demonstrate the potential role of androgen in the regulation of TSPY gene expression
- Demonstrate an aberrant expression of TSPY may be detrimental for normal development

## REPORTABLE OUTCOMES

- Demonstration of a preferential elevated expression of TSPY in human prostate cancer and aberrant splicing of TSPY transcripts (and encoded proteins) in prostate cancer. These results raise the possibility that these variant/abnormal TSPY proteins may be involved in oncogenic activities in humans.

Lau Y-FC, Lau HW and Kömüves LG (2003). Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. Cytogen Genome Research, in press.

- Establishing the preliminary evidence for TSPY gene as a cell cycle regulator and promoter of cell proliferation in cultured cells and tumor formation in animals.

Liu XX and Lau Y-FC (2003). Over-expression of the Y-chromosome gene, TSPY, potentiates cell proliferative activities in vitro and in vivo. In preparation.

- Defining the cyclin B binding domains for TSPY. This study establishes the probable mechanism of cell cycle regulation by TSPY.

Li YM, Kido T and Lau Y-FC (2003). TSPY domain important for binding to the mitotic cyclin B, a possible mechanism of cell cycle regulation by the Y chromosome. In preparation.

## CONCLUSION

The initial rationale for the present project is that the Y chromosome is a male-specific chromosome harboring genes important for the physiology of men. Any mutations or abnormal expression of one of several of these genes might affect the functions of male-specific organs, leading to diseased states, such as infertility and cancer. Through the support of the present grant award, we have systematically examined the expression of 31 relevant genes on this chromosome in prostate cancer samples and have identified the TSPY gene to be a cardinal candidate gene involved in male-specific cancer. Further studies suggest that over-expression of TSPY potentiates cell proliferation and tumor formation in nude mice. Our efforts in the past year demonstrate a variation in RNA processing of the TSPY transcripts, resulting in shortened versions of TSPY protein. Protein binding assays show that TSPY binds to the mitotic cyclin B, probably through its NAP-1/SET homologous domain. These findings substantiate the notion that TSPY participate in cell cycle regulation. Its interaction with the cyclin B may play a key mechanism for it to participate in such cell proliferation process.

## SO WHAT

Uncontrolled cell proliferation is a key characteristic of tumor growth. The elevated levels of TSPY expression in prostate cancer and its potentiation of cell proliferation suggest that this Y chromosome gene may be involved in cell cycle regulation. The possible involvement of the TSPY gene in prostatic oncogenesis suggests that the Y chromosome may be involved in prostate cancer

development or progression. Understanding its mechanism contributing to oncogenesis should be significant in establishing the etiology and designing therapeutic strategies for this significant disease in men.

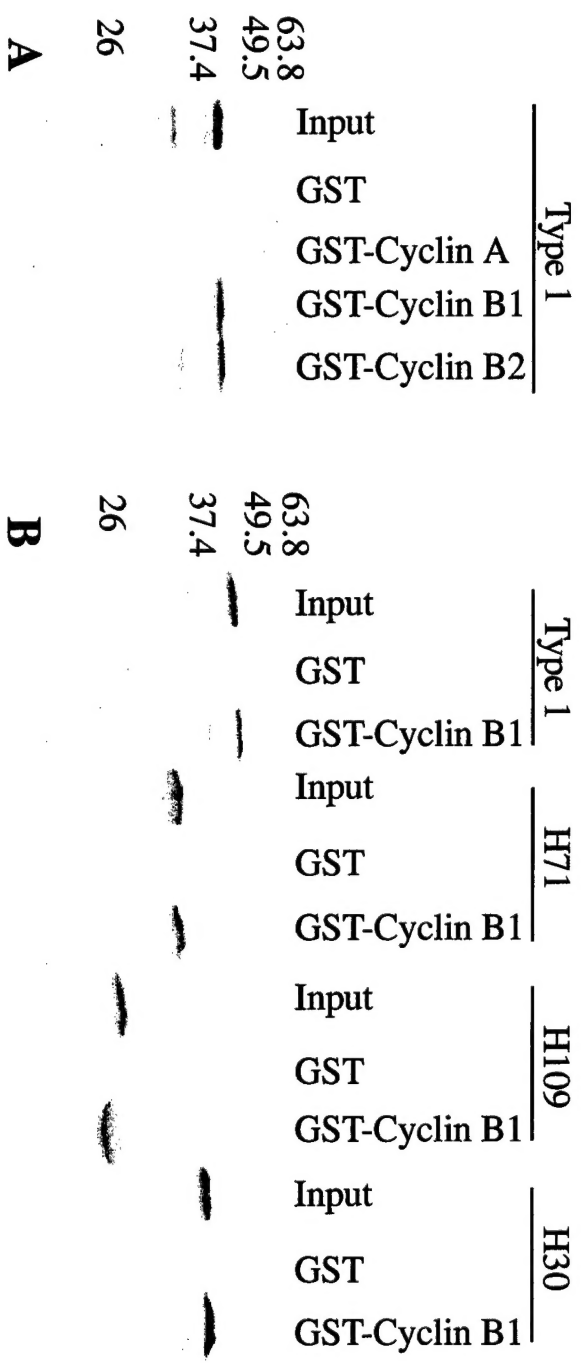
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# **APPENDIX COVER SHEET**

**FIGURES 1-3**



**Figure 1.** GST or GST-cyclin fusion proteins were induced in BL21(DE3) bacterial host by 1mM IPTG, then purified with affinity chromatography using glutathione sepharose. TSPY variants were synthesized by coupled transcription and translation reaction in the presence of <sup>35</sup>S-methionine. GST-pulldown binding assay was performed with 40% TnT labelled products were used for each binding reaction at 4°C for 3 hours, washed 3 X with binding buffer. The bound proteins were then analyzed by SDS-PAGE autoradiography. A. Type 1 labelled-TSPY protein as a probe. The baits were cyclin proteins from *Xenopus*. B. Type 1, H71, H109, H30 were used as labeled TSPY probes. GST-human cyclin B1 fusion protein was used as a bait for the pull-down assays. Size markers are in kDa.

**Figure 2. TSPY Variant Proteins**

Type 1	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	V	C	A	S	A	K	E	G	T	A	F	R	M	E	A	V	Q	E	G	A	50
Type 2	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	V	C	A	S	A	K	E	G	T	A	F	R	M	E	A	V	Q	E	G	A	50
Type 3	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	V	C	A	S	A	K	E	G	T	A	F	R	M	E	A	V	Q	E	G	A	50
H71	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H104	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H109	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H106	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H80	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	V	C	A	S	A	K	E	G	T	A	F	R	M	E	A	V	Q	E	G	A	50
H30	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	V	C	A	S	A	K	E	G	T	A	F	R	M	E	A	V	Q	E	G	A	50
H51	1	MRPEGS	LT	YR	VP	ER	LQ	GF	CG	VG	RAA	Q	AL	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29

Type 1	51	GVSEQA	AL	GE	EA	VL	LL	DD	IM	AE	-----	VEV	VA	EE	EG	L	VER	REE	AQ	PR	Q	94
Type 2	51	GVSEQA	AL	GE	EA	VL	LL	DD	IM	AE	-----	VEV	VA	EE	EG	L	VER	REE	AQ	PR	Q	94
Type 3	51	GVSEQA	AL	GE	EA	VL	LL	DD	IM	AE	VEV	VA	EE	EG	L	VER	REE	AQ	PR	Q	100	
H71	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H104	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H109	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H106	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H80	51	GVSEQA	AL	GE	EA	VL	LL	DD	IM	AE	VEV	VA	EE	EG	L	VER	REE	AQ	RA	Q	100	
H30	51	GVSEQA	AL	GE	EA	VL	LL	DD	IM	AE	-----	VEV	VA	EE	EG	L	VER	REE	AQ	RA	Q	94
H51	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	

Type 1	95	QAVPGP	GP	MP	TP	ES	AL	EL	LA	VQ	VE	LP	NA	QA	KA	FS	RQ	RE	KM	ERR	RR	KPH	144
Type 2	95	QAVPGP	GP	MP	TP	ES	AL	EL	LA	VQ	VE	LP	NA	QA	KA	FS	RQ	RE	KM	ERR	RR	KPH	144
Type 3	101	QAVPGP	GP	MP	TP	ES	AL	EL	LA	VQ	VE	LP	NA	QA	KA	FS	RQ	RE	KM	ERR	RR	KPH	150
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H104	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	40	
H109	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H106	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	29	
H80	101	QAVPGP	GP	MP	TP	ES	AL	EL	LA	VQ	VE	LP	NA	QA	KA	FS	RQ	RE	KM	ERR	RR	KPH	150
H30	95	QAVPGP	GP	MP	TP	ES	AL	EL	LA	VQ	VE	LP	NA	QA	KA	FS	RQ	RE	NM	ER	TR	KPH	144
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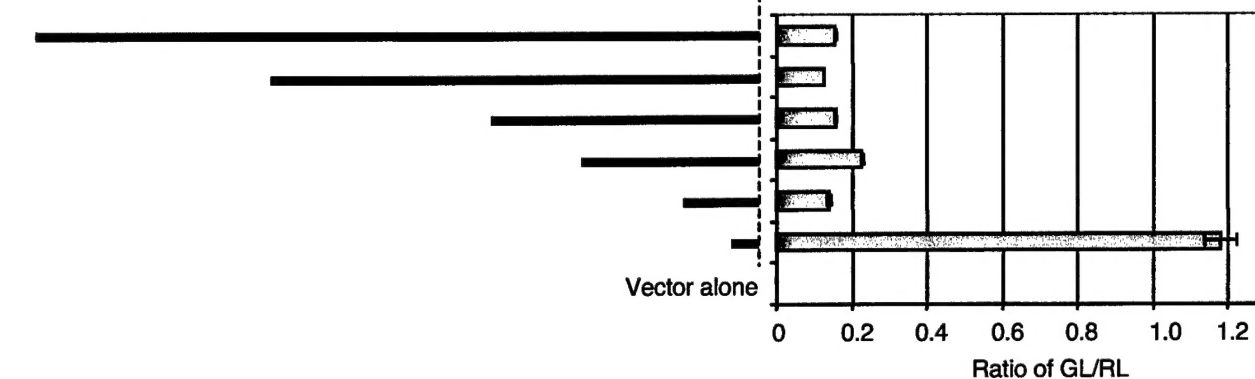
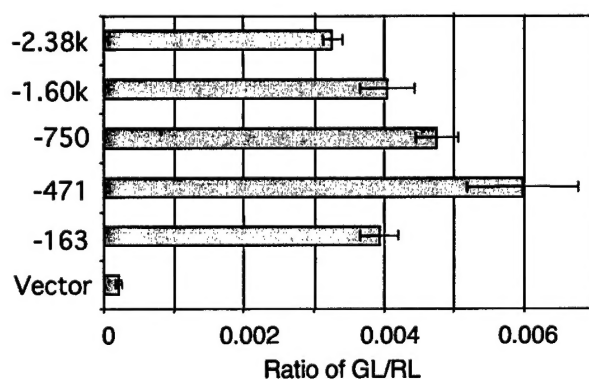
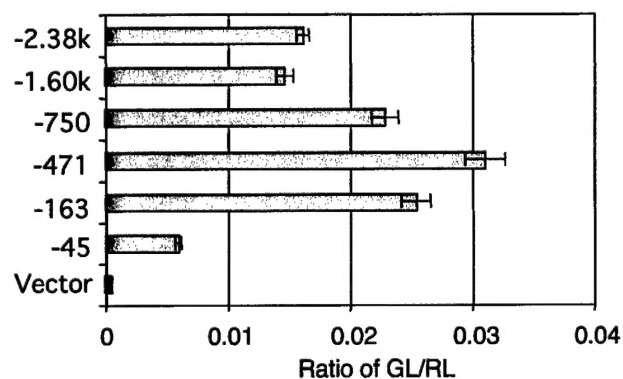
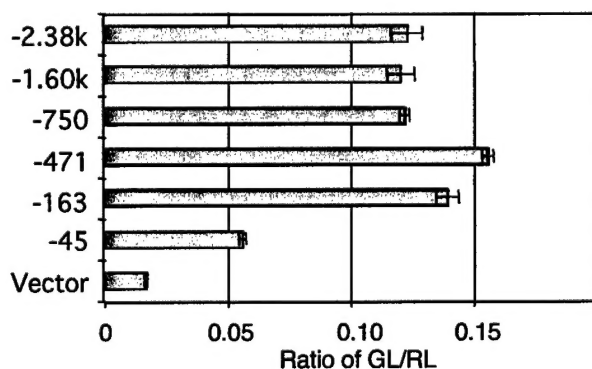
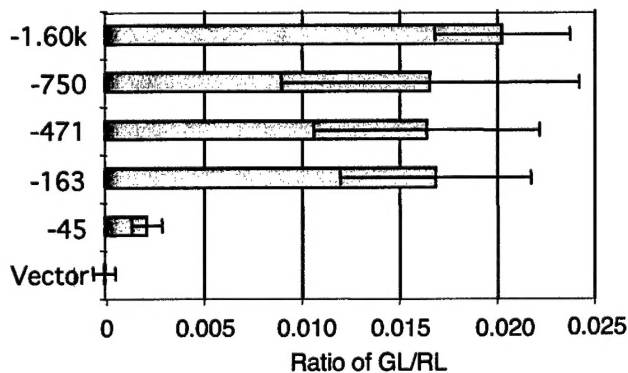
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Type 2	145	LDRRGA	VI	QS	VP	GF	WA	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	194
Type 3	151	LDRRGA	VI	QS	VP	GF	WA	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	200
H71	58	LDRRGA	VI	QS	VP	GF	WA	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	107
H104	41	LDRRGA	VI	QS	VP	GF	WA	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	90
H109	29	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	55		
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H30	145	LDRIGA	VI	QS	VP	GF	WP	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	194
H51	58	LDRRGA	VI	QS	VP	GF	WA	NV	IA	NH	PQ	MS	AL	IT	DE	DE	D	ML	S	Y	M	S	L	E	V	E	E	E	KH	107

Type 1	195	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	244
Type 2	195	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	244
Type 3	201	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	250
H71	108	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	157
H104	91	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	140
H109	56	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	105
H106	56	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	105
H80	201	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	G	D	R	W	L	P	G	W	V	E	G	R	W	V	D	H	250
H30	195	PVHLYK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	244
H51	108	PVHLCK	IM	LF	FR	SN	PF	Q	N	K	V	IT	KE	Y	L	V	N	I	T	E	Y	R	A	S	H	S	T	P	I	E	W	P	D	Y	E	157

Type 1	245	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	IL	CK	DL	WR	N	P	L	Q	Y	Y	K	R	M	K	P	294
Type 2	245	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	SP	DR	SY	VR	T	C	G	A	I	P	C	N	T	T	R	294
Type 3	251	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	IL	CK	DL	WR	N	P	L	Q	Y	Y	K	R	M	K	P	300
H71	158	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	IL	CK	DL	WR	N	P	L	Q	Y	Y	K	R	M	K	P	207
H104	141	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	IL	CK	DL	WR	N	P	L	Q	Y	Y	K	R	M	K	P	190
H109	106	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	IL	CK	DL	WR	N	P	L	Q	Y	Y	K	R	M	K	P	155
H106	106	AYRRRH	HN	SS	LN	FF	NW	FS	DH	NF	AG	SN	KI	AE	SP	DR	SY	VR												

**A**

Sph1                      Mlu1                      Sac1                      Sph1  
 -2.38k                      -1.60k                      -750                      -471                      -163                      -45                      +44

**B. COS7****C. GC1spg****D. GC2spd****E. PC3****F. HeLa**

**Figure 3.** 0.3 $\mu$ g of pGL2-TSPY promoter or pGL2-basic was transfected into cultured cells with 15ng of pRL-TK (24 well plate). Transfection was performed by Fugene6 (Roche). After transfection, cells were incubated for 2 days. The promoter activity was measured by a dual luciferase assay kit (Promega). Values mean the ratio of tested promoter activity (GL)/internal control activity (RL).